PROJECT CHARGE: 2106

PROJECT TITLE: APPLIED TECHNOLOGY

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PROJECT GROW

Together with W. Mutter's Division and outside consultants the Puff Profile Analyzer has been very carefully examined to verify its operation. No major problems were found and the unit has been used in a demonstration of the Barclay dilution mechanism to outside consultants.

NATURALLY OCCURRING DENITRIFICATION

The series of Fed-batch runs in the pilot plant with the mixed culture innoculum derived from a phauxostat have been completed. They are currently being analyzed to show the effect of different SEL feed rates and differing seed levels of innoculum. Extremely good reproducibility was obtained with pasturized SEL and accurately controlled temperature.

A presentation on the results so far was made to Helmut Gaisch and Deiter Schultes of P.M. Europe. Several meetings were held with them to evaluate additional information needed to make this a viable process.

A project was undertaken in collaboration with B. Semp (Mfg.) to characterize the organism primarily responsible for denitration, to define the optimal innoculum and to obtain the data necessary to design an innoculum feed line.

The results of the biochemical characterization tests furnished by ATCC on the submitted isolates were examined. A comparison showed that ATCC-1 was 85% biochemically similar to a later isolate from the Fed-batch reactor identified by ATCC as <u>Bacillus circulans</u>. The matches between ATCC-1 and other identified isolates were weak. A culture of <u>Bacillus circulans</u> was grown up and was shown to reduce NO_3^- to N_2^- in a phauxostat.

Collaborative work with B. Semp was also begun on a synthetic start up medium. V. Malek had shown that ATCC-1 can be successfully started in a 10% solution of SEL in water. To avoid the problems inherent in the storage of large volumes of SEL some SEL was concentrated to 70% dissolved solids. This concentrate minimized both volume and spoilage problems. A medium containing glucose and NH4NO3 was prepared. Shaker flasks containing this medium as well as tobacco solubles ranging in concentration from 0.05 to 1% as added from the

concentrate were innoculated with ATCC-1. Growth was observed in all flasks and appeared to be proportional to the concentration of tobacco solubles over the range tested.

HEAT TREATMENT OF TOBACCO MATERIALS

the spatial distribution of density.

Work has continued on developing the phase diagram of bright, burley and oriental tobacco. A problem with Karl Fisher determinations of the expanded samples has been solved.

Oriental tobacco has been heated at low temperatures (135-200°F) for varying lengths of time with no apparent pattern for CV increases. Work is continuing at higher temperatures.

ADDITIONAL PROJECTS

It was found that there was insufficient contrast and regularity of the dark bands on the BL sheet to allow accurate quantifications of their intensity and frequency using the rotating wheel and the Fast Fourier Transform oscilliscope. A system is being set up to scan any future sample for

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